Evaluation of MicroRNA-451a Expression in Lupus Nephritis

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ABSTRACT

The pathogenesis of Lupus Nephritis (LN) is still unclear, causing difficulties in diagnosis and therapeutic management. Recent studies have suggested that microRNAs (miRNAs) may play an important role in the pathogenesis of LN. However, the role of miRNA-451a in LN has not been widely studied and known, and there is controversy in the results of studies that have been done. This study aimed to analyze the expression of miRNA-451a in LN and lupus without nephritis and to evaluate its correlation with hemoglobin levels, leukocyte count, leukocyturia, and erythrocyturia. Samples from 45 lupus participants were collected, consisting of 22 with LN and 23 without nephritis. The expression of microRNA 451a was determined by reverse transcriptase real-time PCR. Renal disorder criteria based on ACR criteria. Statistical analysis using Mann-Whitney, Spearman correlation, ROC curve, and table 2X2. MiRNA-451a expression in LN (median 0.31; range 0.06-7.21) was lower than in lupus without nephritis (median 1.31; range 0.07-13.26) with a significant difference (p=0.041). The cut-off value for comparing the two groups was 0.7531 with an accuracy of 75%. The correlation between miRNA 451a expression and hemoglobin levels, blood leukocyte count, erythrocyturia, and leukocyturia showed no significant correlation (p > 0.05). MicroRNA-451a expression in lupus nephritis was lower than in lupus without nephritis.

Keywords: MicroRNA-451a expression, lupus, lupus nephritis

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with diverse clinical manifestations and is able to disrupt various organs.¹,² Globally, the incidence of SLE varies between races, but women of childbearing age (15 to 44 years) are known to be the most commonly affected.³

Progressive and irreversible organ damage poses a challenge in SLE management. One of them is renal damage found in more than 50% of SLE patients in the first year after being diagnosed with lupus.⁴⁻⁵ Continuous renal impairment may progress into Lupus Nephritis (LN), significantly impacting patient morbidity and mortality.⁶,⁷

The variety of clinical manifestations and the unclear pathogenesis of the disease influence difficulties in diagnosing and managing SLE.²,⁸ In recent years, the clinical relevance of microRNA (miRNA) in the pathogenesis of SLE has been discovered.⁹⁻¹² Micro-RNA is a small non-coding RNA (ribonucleic acid) consisting of 19 to 25 nucleotide bases found in various cells and can affect the translation of 60% of mRNA (messenger RNA).¹¹,¹² MicroRNAs that play a role in SLE renal damage include miRNA-155, miRNA-150, and miRNA-451a.⁸ The microRNA thought to play a role in LN, and SLE pathogenesis is miRNA-451a. MicroRNA-451a is also known to affect hematopoiesis, especially erythroid and non-erythroid cells.¹³ Therefore, this study evaluates the correlation between miRNA-451a expression with hemoglobin levels and blood leukocyte count. miRNA-451a expression with renal function parameters such as creatinine or Glomerular Filtration Rate (GFR) were not correlated, because in mild to moderate renal damage, creatinine has poor sensitivity.¹⁴ Based on research by Satoh et al. Anti-Su autoantibodies were found to play a role in lupus, targeting Argonaute 2 (Ago2) protein damage.¹⁵ Disruption of Ago2 as miRNA-451a biogenesis protein will reduce the production of miRNA-451a. The results of Tan et al. study showed that decreased miRNA-451a expression in the exosomal serum of SLE patients was correlated with lupus disease activity (SLEDAI) and renal damage.¹⁶ Therefore this study compares the miRNA-451a expression between LN (the renal damage group) and lupus without nephritis, and the cut-off of MicroRNA-451a...
expression between the two groups. Lupus disease activation in renal is evaluated by the correlation between miRNA-451a expression, with hematuria and leukocyturia.

Hong et al. state that the inhibition of miRNA-451a was found to reduce the number of anti-dsDNA antibodies, recover defects from Treg cells, reduce B cell activation and proliferation, and was able to show improvements in the spleen and kidney of SLE mice models. The studies above show that the expression of miRNA-451a in SLE and its manifestations is still debated whether its effect is beneficial or detrimental to the renal system, making it interesting to study as it is the first study on Indonesia’s population.

METHODS

A cross-sectional study from January 2021 to June 2022 at Dr. Saiful Anwar General Hospital, Malang. The inclusion criteria were female patients with lupus, based on the Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria, aged 18 to 65. The exclusion criteria were patients suffering from active infectious disease, diabetes, or renal failure. The requirements of LN participants according to American College of Rheumatology (ACR) criteria, consisted of lupus patients with persistent proteinuria or greater than 500 mg per day (or more than 3+ on dipstick examination), urine sediment abnormality, or renal biopsy results indicating LN.

The random venous blood samples will be taken for miRNA-451a examination obtained from the Ethylene Diamine Tetra Acetic Acid (EDTA) tube used to collect the plasma. The complete blood count test was done using a Sysmex XN-1000 Hematology Analyzer with flow cytometry method. The plasma samples were stored at -80°C until the RT-PCR examination. The first step was the extraction of RNA with the RNAqueous-Micro kit, followed by the cDNA Reverse Transcription kit to perform reverse transcription. The next step was to quantify the expression of miRNA-451a using Reverse Transcription-Real Time Polymerase Chain Reaction (RT-qPCR). U6 was used as a housekeeping gene to normalize the expression of miRNA-451a. The results of this study showed that the age of patients with lupus consists mostly of females of childbearing age. All participants in this study were female, due to estrogen role in SLE that may interfere with the result of the study.

The minimum sample was 18 for each group. The ethical committee of Dr. Saiful Anwar General Hospital, Malang, approved this study (ethical number 400/247/K.3/302/2020).

Data was analyzed using IBM SPSS version 25 with a 95% confidence interval and α=0.05. The statistical test was declared significant if p <0.05. Normality and homogeneity tests were performed using the Shapiro-Wilk and Levene test. MicroRNA-451a has abnormal (group 1=0.001, 2=0.000) and heterogenous data (0.018), and therefore Mann-Whitney test was done. Then, the Spearman test was done to evaluate the correlation between miRNA-451a and Hb level, blood leukocyte count, leukocyturia, also hematuria. The cut-off of MicroRNA-451a expression between the two groups was made by ROC curve and 2x2 table to find the accuracy of miRNA-451a.

RESULTS AND DISCUSSIONS

In this study, the characteristics of participants can be seen in Table 1 with the criteria of age, hemoglobin level, leukocyte count, platelet count, urine erythrocyte count, and urine leukocyte count. There were a total of 45 participants, 23 in lupus without nephritis and 22 in lupus nephritis (p<0.041).

The difference in the expression of miRNA-451a is shown in Figure 1 with a lower value in the LN group compared to lupus without nephritis. The ROC result is shown in Figure 2 with the ROC curve value of 0.801. Based on cut-off 0.7531 the sensitivity was 69.6%, the specificity was 72.7% and the accuracy was 75%. There were seven samples of false positives and six samples of false negatives.

Then, the correlation value between miRNA-451a expression with Hb levels, blood leukocyte counts, leukocyturia, and erythrocyturia can be seen in Figure 3.

Figure 1 showed the miRNA-451 expression in LN patients showed lower expression than in lupus without nephritis (expression of miRNA-451a in lupus without nephritis was 1.31 (0.07-13.26); meanwhile the expression of miRNA-451a in LN was 0.31 (0.06-7.21), p=0.041).

The results of this study showed that the age of patients with lupus consists mostly of females of childbearing age. All participants in this study were female, due to estrogen role in SLE that may interfere with the result of the study.

The hemoglobin levels, leukocyte, and platelet count in this study did not show significant differences between the LN and lupus without
Table 1. Characteristics of research participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lupus without Nephritis</th>
<th>Lupus Nephritis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (Range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.87 (7.80)</td>
<td>23 (18.0-45.0)</td>
<td>32.64 (10.28)</td>
</tr>
<tr>
<td>Hb Level (g/dL)</td>
<td>11.93 (1.99)</td>
<td>12.5 (7.40-14.10)</td>
<td>11.71 (1.70)</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>6.88 (2.60)</td>
<td>6.47 (3.64-14.70)</td>
<td>8.30 (3.45)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>264.04 (116.57)</td>
<td>270 (48.0-564.0)</td>
<td>275.32 (69.29)</td>
</tr>
<tr>
<td>Urine erythrocyte count (HPF)</td>
<td>5.27 (14.90)</td>
<td>0.8 (0.10-69.30)</td>
<td>26.63 (68.23)</td>
</tr>
<tr>
<td>Urine leukocyte count (HPF)</td>
<td>10.97 (37.87)</td>
<td>1.8 (0-184.10)</td>
<td>11.38 (16.52)</td>
</tr>
</tbody>
</table>

Note: *The median value of the study subjects was significant between the groups of LN and lupus without nephritis (p-value < 0.05). The median was used in all characteristics due to normality value, and the Mann-Whitney test was used as a comparison test. HPF (High Power Field)

Figure 1. Differences in microRNA-451a expression in the LN group compared to lupus without nephritis

Figure 2. The ROC curve of miRNA451-a expression to differentiate lupus patients without nephritis and LN was 0.801 (95% confidence interval, 0.515–0.8341; p < 0.05) nephritis groups; also, there was no correlation between miRNA 451a expression and hemoglobin levels or leukocyte count. This is possibly the inflammation due to lupus or miRNA 451a not suppressing the hematopoiesis system in the LN groups. Additionally, not all lupus patients have hematological abnormalities. 1,8

The data of hematuria and leukocyturia showed a significant difference between LN and lupus without nephritis groups. Still, there was no correlation between 451a miRNA expression and urine erythrocyte count or leukocyte count. Lupus patients with increased urinary protein are also suggested for increased lupus activity in the renal domain. The increasing disease activity is generally characterized by the presence of hematuria and leukocyturia in addition to proteinuria. Kidney damage in lupus will trigger the release of NET, which is a source of self-antigen for the production of autoantibodies. NET can also trigger inflammation leading to endothelial damage and IFN-α production by dendritic cells, further exacerbating inflammation and autoimmune conditions. Furthermore, the complement system can trigger inflammation by attracting leukocytes to the renal or by damaging the renal directly through the formation of the Membrane Attack Complex (MAC), resulting in hematuria. 20

This study showed that the lupus group (without nephritis) had higher expression of miRNA-451a, which had a median value of 1.31 (0.07-13.26) while the LN group showed a result of 0.31 (0.06-0.21). Based on the results of statistical tests, the two
groups showed a significant difference \( p < 0.05 \). It can be concluded that there is a decrease in miRNA-451a expression in LN patients compared to lupus without nephritis patients. According to Satoh et al., this study results can explain that Anti-Su autoantibodies were found to play a role in lupus with protein Ago2 as the target of damage.

The decrease of Ago2 as a miRNA-451a biogenesis protein will result in a decrease of miRNA-451a production.\(^{17}\) This results in the emergence of inflammation through the TLR4, MYD88, IRAK 1/2, TRAF6, and TAK pathways, which then have an impact on the AP1 and NF-κB pathways that affect the cytokines TNF and IL-8.\(^{21}\)

The study of Tan et al. showed that decreased expression of miRNA-451a in the exosomal serum of SLE patients was correlated with lupus disease activity (SLEDAI) and renal damage.\(^{16}\) Studies of miRNA-451a in SLE are still scarce, and studies of miRNA-451a in patients with other autoimmune diseases have shown low miRNA-451 expression associated with tissue inflammation. A study by Murata et al. on Rheumatoid Arthritis (RA) patients showed that miRNA-451 expression in neutrophils isolated from RA patients had a significantly lower value than healthy controls. The study then continued by systematically administering miRNA-451 in experimental animals, and it was found to reduce neutrophil infiltration in areas of local inflammation dramatically. Overexpression of miRNA-451 significantly suppressed neutrophil migration to areas of inflammation, because overexpression of miRNA-451 will suppress the phosphorylation of p38 MAPK through the 14-3-3 pathway, which is the target of miRNA-451, and via Rab5a. The study also stated that in mice, the administration of miRNA-451 therapy reduced the severity of arthritis and the amount of cell infiltration into the inflammatory area.\(^{22}\)

However, the following studies on miRNA-451a expression showed different results from this. Research by Hong et al. showed that inhibition of miRNA-451a was able to reduce the number of anti-dsDNA antibodies, restore defects in Treg cells, reduce B cell activation and proliferation, and was able to show improvements in the spleen and renal of SLE mice models.\(^{23}\) The research of Cheng et al. also stated that there was an increase in the expression of miRNA-451a in the spleen and thymus of SLE model mice, and the decreased expression of miRNA-451a was able to indirectly reduce spleen enlargement, improve proteinuria and reduce immune complex deposits, where miRNA deficiency -451a showed improvement of CD4+CD69+ T-cells.

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**Figure 3.** (A) Correlation between MicroRNA-451a and hemoglobin levels \( p = 0.123 \ r = -0.223 \); (B) Correlation between MicroRNA-451a and leukocyte count \( p = 0.722 \ r = 0.055 \); (C) Correlation between MicroRNA-451a and erythrocyturia \( p = 0.792 \ r = -0.040 \); (D) Correlation between MicroRNA-451a and leukocyturia \( p = 0.681 \ r = -0.063 \). The correlation was done using the Spearmen test.
and reduction of serum cytokines such as IL17a and IL-4.\(^\text{25}\)

This study showed that miRNA-451a with cut-off 0.7531 has a sensitivity of 69.6%, specificity of 72.7%, and accuracy of 75%. This study’s results matched Tan et al. study with a previous sensitivity of 70% and specificity of 100% on patient lupus with renal damage from patient lupus without renal damage. However, with the cut-off of 0.7531, this study showed some false positive and negative results. These findings may be caused by the different amounts of Anti-Su autoantibodies that can influence the miRNA-451a expression.\(^\text{15,16}\)

Some of the results of the studies above indicate that the expression of miRNA-451a in renal disorders in lupus and its manifestations in LN is still debated whether it is beneficial or detrimental. However, this study’s results suggest that decreased miRNA-451a expression is associated with renal damage in LN patients. Based on this, further research is needed with a larger number of samples and different populations to determine the proper role of miRNA-451a in LN.

This study has several limitations, namely, the miRNA-451a examination sample was only taken in one hospital and the number was limited.

CONCLUSIONS AND SUGGESTIONS

Based on our study results, it can be concluded that MicroRNA-451a expression in LN was lower than lupus without nephritis. Further research is needed regarding the role of microRNA-451a in SLE patients with LN in different populations.

REFERENCES